



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Boyce et al.

EXAMINER: Prebilic, P.

SERIAL NO.: 09/543,268

GROUP ART UNIT: 3738

FILED: April 5, 2000

DOCKET: 285-79 CON

FOR: OSTEOIMPLANT AND
METHOD FOR
ITS MANUFACTURE

Commissioner for Patents
Washington, D.C. 20231

COMBINED DECLARATION OF TODD M. BOYCE
AND ALBERT MANRIQUE UNDER 37 C.F.R. § 1.131

Sir:

We, Todd M. Boyce, Ph.D., and Albert Manrique, declare and say as follows:

1. Todd M. Boyce is a Senior Scientist with Osteotech, Inc., the assignee of record of the subject patent application, and a named inventor therein.
2. Albert Manrique is a Senior Research Scientist with Osteotech, Inc., the assignee of record of the subject patent application, and a named inventor therein.
3. In the Office Action mailed April 2, 2003, the Examiner has maintained the rejection of Claims 1-7, 9-21, 23-43, 45-61, 63-80 and 82-134 of the subject patent application under 35 U.S.C. § 102(e) as unpatentable over Boyce et al. U.S. Patent No. 5,899,939 ("Boyce et al.") which issued on May 4, 1999 on original application Serial No. 09/009,997 filed January 21, 1998.
4. In the April 2, 2003 Office Action, in discussing a declaration previously submitted with respect to the Boyce reference, the Examiner states at page 5 as follows:

Claim 1 is not commensurate with the scope of the invention shown in the declaration in that claim 1 is drawn to a broader invention. It is noted that 4 of the 5 examples in Exhibit A are to sheet forms of the implant, yet claim 1 is drawn to any solid aggregate. The other example (Example 2) is not drawn to "elements bonded through chemical linkages to the surface-exposed collagen" as claimed. In Example 2, there is apparently no chemical bonding. Even though claim 1 is not commensurate with the declaration invention, there is no showing that the different is obvious; see MPEP 715.02, 3rd paragraph (August 2001 edition).

5. The subject application was filed on April 5, 2000 as a continuation of U.S. patent application Serial No. 09/020,205 filed February 6, 1998 which issued as U.S. Patent No. 6,123,731 on September 26, 2000.

6. We make this Declaration jointly under 37 C.F.R. § 1.131 in order to present a showing of facts evidencing the making of the claimed invention in this country prior to the January 21, 1998 filing date of the aforesaid Boyce et al. application.

7. All of the acts described hereinafter took place in the United States.

8. Annexed Exhibit A, of which Todd M. Boyce is the author, is a true copy of a memo and accompanying drawings, redacted to remove references to dates and non-relevant subject matter, that was prepared by Todd M. Boyce prior to January 21, 1998.

9. Albert Manrique was party to discussions which led to the concepts memorialized in Exhibit A.

10. Exhibit A describes embodiments of the invention of amended Claim 1 herein. This Exhibit conclusively shows that prior to the January 21, 1998 filing date of the application underlying the grant of Boyce et al. U.S. Patent No. 5,899,939, the invention herein had been completed in this country.

11. As set forth on the second and third pages of Exhibit A, references are made to cross-linking of collagen, including by chemical or enzymatic reaction which encompass the chemical linkages of Claim 1.

12. Example 2 of Exhibit A exemplifies a solid aggregate, i.e., a pellet, made of demineralized elongate bone fibers in which individual demineralized bone fibers are bonded to each other through chemical linkages between their surface-exposed collagen. As such, the pellet of Example 2 of Exhibit A is an embodiment of Claim 1 of the subject application.

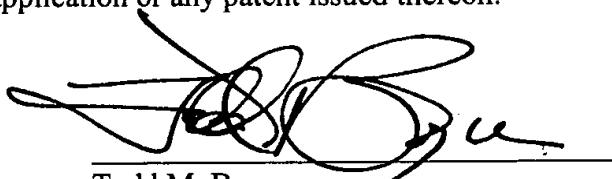
13. The pellet of Example 2 of Exhibit A was produced by demineralizing bone followed by its placement "in polyethylene glycol diglycidal ether for 12 hours at room temperature." As set forth on page 3 of Exhibit A, "chemicals which act as collagen cross-linkers include: ...polyepoxy compounds such as...polyethylene glycol diglycidal ethers...." This clearly encompasses the solid aggregate of Claim 1 wherein the demineralized bone-derived elements are bonded to each other through chemical linkages between their surface-exposed collagen.

14. Moreover, Examples 1, 3, 4, and 5 combine multiple layers of sheets (Examples 1, 4 and 5) or bone cubes in combination with bone slices (Example 3). Cross-linking of surface exposed collagen takes place by placing the demineralized bone into 10% neutral buffered formalin (Examples 1, 3 and 5) or by treating the bone with transglutaminase (Example 4). As all of these examples encompass cross-linking of multiple layers or pieces of bone, we respectfully submit the resulting implant would encompass the solid aggregate of Claim 1.

15. Alternatively, we believe the claimed invention would be obvious to one skilled in the art who reviewed Exhibit A, especially in view of the foregoing references to pellets, multiple layers of bone sheets and bone cubes in combination with bone slices, which were all made from demineralized bone bonded through chemical linkages between surface-exposed collagen by exposure to chemicals or enzymes which acted as collagen cross-linkers.

16. We each further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: May 6, 2003



Todd M. Boyce

Dated: 5/6, 2003



Albert Manrique

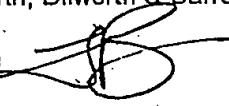
MRB:mg



Memorandum

DATE:

TO: Peter Dilworth, Dilworth & Barrese

FROM: Todd Boyce 

RE: 285-79: Osteoimplant and method for its manufacture

(VIA FAX; Originals to follow by post)

Attached are the background information materials that I promised, regarding the second application, 285-79. In this FAX transmittal are included:

- A document describing materials for claim 5, background information on cross-linking of collagen, the use of glycerol to maintain hydration, and five example descriptions.
- Hand-drawn pictures to accompany the five examples.

In the copy that I send to you by mail, I'll also include the articles by Lewandrowski, Jurgensen, and an article that one of my co-workers found on fibrin glues used with hydroxyapatite to make implants for bone replacement.

I've tried to include information that I thought you might need. Please fell free to call me (732-544-6235) if I have missed important points, or if I have been unclear in any of this. Nelson and I look forward to moving ahead with this application, and to working with you further as it develops.

97_1105a

A

285-79: List of materials for Claim 5:

The group including glycerol, any tanning agent, substances imparting radioopacity, metallic meshes, hydrophilic components, antibiotic and/or antiviral agents, activated protein-based binders, lysine-rich proteins, allogenic or xenogenic serum albumin, allogenic or xenogenic fibrin, thrombin or other blood or serum elements, allogenic or xenogenic collagen, collagen glues, fibrin glues, polymethyl methacrylate, cyanoacrylate, autologous bone cells or stem cells, hydroxyapatite or other calcium-phosphate-based minerals, calcium sulfate, bioglass, cancellous bone, any of the family of bone morphogenic proteins, transforming growth factor beta, Insulin-like growth factor, calcium chloride, glucose or other sugars.

In addition to what we currently have, Claim 1 could also generalize to bone elements which might be either cortical or cancellous, or combined corticocancellous in structure.

The list of bones for claim 11 is given in my correspondence to you on application 285-79.

The list of osteoinductive materials for claim 18 is also given in my correspondence to you re: application 285-79. Osteogenic osteoinductive and osteoconductive materials for claim 24 are also given there.

285-79: Collagen cross-linking

Collagen is a naturally-occurring structural biomaterial, and is a part of connective tissues, including bone, in all vertebrate species. The native collagen molecule is a glycine-rich chain of amino acids, arranged in a triple helix. It is known that collagen-containing tissues may be preserved by cross-linking the collagen molecules together by any of a number of means, including chemical reaction, the application of radiant energy, dehydrothermal treatment, or enzymatic treatment. In each case, exposed functional groups of the collagen amino acids react to form stable intra- and inter-molecular bonds. These bonds act to stabilize the tissue against breakdown, and impart additional mechanical strength. These approaches are used in the preparation of biomaterials, in fixation of tissues for histological processing, in tanning of leathers and furs, in embalming, and in other manufacturing

Bone is made of collagen, hydroxapatite mineral, and other non-collagenous proteins. The present invention removes the mineral in bone by treating the bone with acid

solutions, detergents, or chelating agents, and then placing surface-demineralized particles in contact with each other. The assembled construct is then treated with a cross-linking agent or process to cross-link the collagen molecules, providing bonding or adhesion between particles. Furthermore, since acid demineralization is a diffusion-limited reaction, with an advancing reaction front (2), bone-derived particles may be demineralized at the surface, exposing the collagen there, while leaving the inner core of the particle fully mineralized. The depth of demineralization is a function of the strength of the acid solution, the shape of the particle, and the treatment time. (2). Suitable compounds for demineralizing bone include hydrochloric acid, hydrofluoric acid, acetic acid, sulfuric acid, phosphoric acid, hydrobromic acid, hydroiodic acid, hydrosulfuric acid, nitric acid, nitrous acid, perchloric acid, chloric acid, chlorous acid, hypochlorous acid, hypochlorous acid, acetic acid, sulfurous acid, carbonic acid, boric acid, ethylene diamine tetraacetic acid (EDTA). The most useful of these are solutions of hydrochloric acid, acetic acid, and EDTA.

Chemical cross-linking agents usually contain bifunctional or multifunctional reactive groups, which react with functional groups on the amino acids, such as the ϵ -amine functional group of the lysine or hydroxylysine, or the carboxyl functional groups of aspartic and glutamic acids. By reacting with multiple functional groups of different collagen molecules, the reacting chemical creates a reinforcing cross-bridge. Chemicals which act as collagen cross-linkers include: mono- and dialdehydes, including glutaraldehyde and formaldehyde; Polyepoxy compounds such as glycerol polyglycidal ethers, polyethylene glycol diglycidal ethers and other polyepoxy and diepoxy glycidal ethers; Tanning agents including polyvalent metallic oxides such as such as titanium dioxide, chromium dioxide, aluminum dioxide, zirconium salt, as well as organic tannins and other phenolic oxides derived from plants; esterification of carboxyl groups followed by reaction with hydrazide to form activated acyl azide functionalities in the collagen; dicyclohexyl carbodiimide and its derivatives as well as other heterobifunctional cross-linking agents; hexamethylene diisocyanate. Sugars, including glucose, will also cross-link collagen; this is a pathological process which can occur in living diabetic patients. Chemical cross-linking of the exposed collagen will involve exposing the assembled bone-derived pieces to the chemical agent, either by placing it in a solution of the chemical agent, or by exposing it to the vapors of the chemical agent at an appropriate pH and temperature, and for times ranging from minutes to days, depending upon the level of cross-linking desired, and the activity of the chemical agent. The chemical agent is then washed to remove all leachable traces of the chemical.

Glutaraldehyde cross-linked biomaterials have a tendency to over-calcify in the body. Calcification-controlling agents which might be used with aldehyde cross-linking agents include: dimethyl sulfoxide (DMSO), Surfactants, diphosphonates, aminooleic acid, and metallic ions, primarily ions of iron and aluminum.

Physicochemical and physical methods for cross-linking tissues include: Dye-mediated photo-oxidation, irradiation by UV light or microwaves, drying and/or heating, dehydrothermal methods (in which water is slowly removed while the tissue is in a vacuum).

Enzymatic treatment is another way to introduce cross-links between the collagen molecules. Treatment by enzymes such as tissue transglutaminase can catalyze reactions between the gamma carboxylic acid group of amino acids to the amino group of lysine in the collagen molecules. This approach has been used previously to bond cartilage (1), however our application here is a novel use which provides bonding between partially or fully demineralized bone pieces, and imparts substantial mechanical strength to the bonding interface.

285-79: Use of glycerol to maintain hydration and prevent separation of bone elements

Following the creation of an osteoimplant, and for the purpose of storage prior to implantation, the osteoimplant may require free water to be removed, via freeze-drying, or other drying steps. We have found that in this process, residual strains in the mineral-containing bone elements making up the osteoimplant may cause warping of layered constructs, which in turn may produce separation between cross-link bonded components. The addition of an aqueous glycerol-containing solution or other additive helps to maintain hydration of the osteoimplant, and reduces or eliminates this warping effect.

285-79 Examples:

Example 1 (Pilot 28): Slices of compact bone, each approximately 1.5 - 2 mm thick were placed into excess 0.6 N HCl solution for 1.5 hours with constant stirring. The pieces were washed in water for 5 minutes, and soaked for 1.5 hours in BupH phosphate buffered saline (Pierce, Catalog #28372). The slices were stacked into layers, and were clamped together. The clamped construct was then placed into a solution of 10% neutral buffered formalin for 48 hours to cross-link the exposed collagen surfaces. After cross-linking, the clamp was removed, and the construct was placed in a water bath to rinse in running water for several hours. The construct was cut to shape on a band saw, and then placed in an excess aqueous solution of glycerol. After seven hours, the excess glycerol solution was removed, and the osteoimplant was freeze-dried.

Example 2 (Pilot 11F): Elongate bone fibers were milled from cortical bone, and were fully demineralized in excess 0.6N HCl solution. These fibers were washed with water, and soaked in an aqueous solution of glycerol. Fully mineralized fibers were added, and the solution was stirred and left for 12 hours at room temperature. The solution containing the soaked mineralized and demineralized fibers were poured through a micron sieve to recover the fibers. The fibers were then pressure-treated to 10,000-50,000 psi in a press for 15 minutes, and were then heated for 2 to 12 hours at 37-55 degrees C. The resulting osteoimplant pellet was freeze dried, and placed in polyethylene glycol diglycidal ether for 12 hours at room temperature.

Example 3 (pilot 14): Human cortical bone slices, approximately 1 mm thick by 7mm wide by 5cm long, were treated for 10 minutes in 0.6 N HCl to expose surface collagens. Human cancellous bone cubes, 1cm x 1 cm x 1cm, were treated to expose surface

collagens at the outer borders of the cubes. All slices and cancellous bone cubes were washed in water. The pieces were assembled together with cortical slices bordering the cancellous blocks, and clamped into place. The construct was then placed into a solution of 10% neutral buffered formalin for 3 hours to crosslink the exposed collagens. The resulting osteoimplant was then washed in water, and cut to size on a band saw.

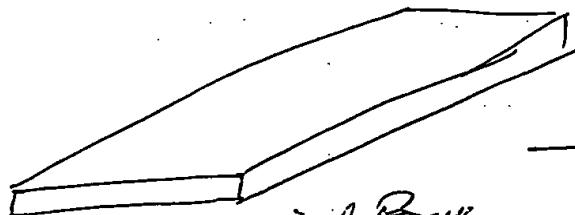
Example 4 (Pilot 21): Human bone segments, approximately 1mm thick were surface demineralized for 15 minutes in 0.6N HCl, then washed in running water. Tissue transglutaminase was reconstituted to give a 1mg/ml solution. For each layer of the construct, the surface was blotted dry, then 40 μ l/cm² area of the tissue transglutaminase was applied to one side of a demineralized bone slice, and an equivalent volume of 0.1M CaCl₂ solution was applied to the mating surface of the next bone slice. This was repeated sequentially. The resulting construct was clamped and placed into a humidity chamber for approximately 30 minutes, then washed in water.

Example 5: Cortical bone slices, approximately 2mm thick, were surface demineralized in 0.6N HCl solution for 1 hour with constant stirring. Bone slices were coated with dry, demineralized bone powder having a particle size of 300 microns or less, and assembled into layers. The construct was clamped into place, and placed into a solution of 10% neutral buffered formalin for 12 hours to permit collagen cross-linking. The resulting osteoimplant was washed in water to remove excess chemicals.

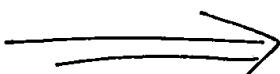
References

1. Jürgensen K, Aeschlimann D, Cavin V, Genge M, Hunziker EB: A new biological glue for cartilage-cartilage interfaces: Tissue transglutaminase. *J Bone Joint Surg [Am]* 79-A:185-193, 1997
2. Lewandrowski K-U, Venugopalan V, Tomford WW, Schomacker KT, Mankin HJ, Deutsch TF: Kinetics of cortical bone demineralization: Controlled demineralization -- a new method for modifying cortical bone allografts. *J Biomed Mater Res* 31:365-372, 1996

285-79 EXAMPLE 1



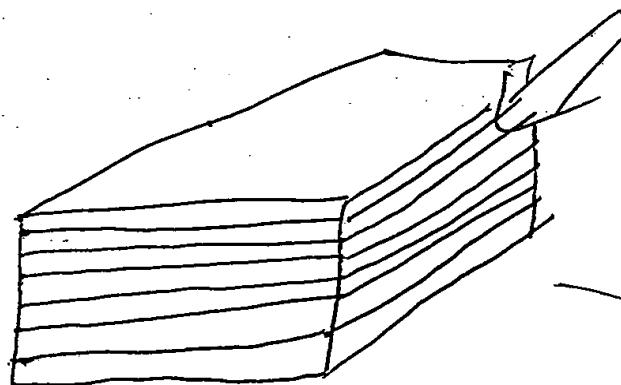
Cortical Bone
strips



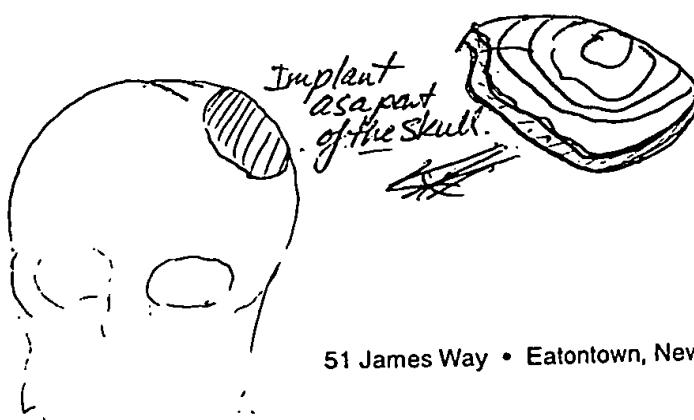
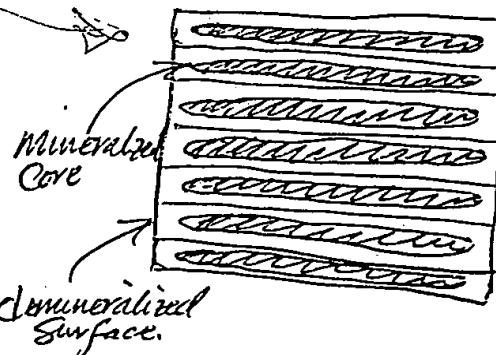
Surface.
Deminerilize
in Acid.



Clamp together
Cross-link.

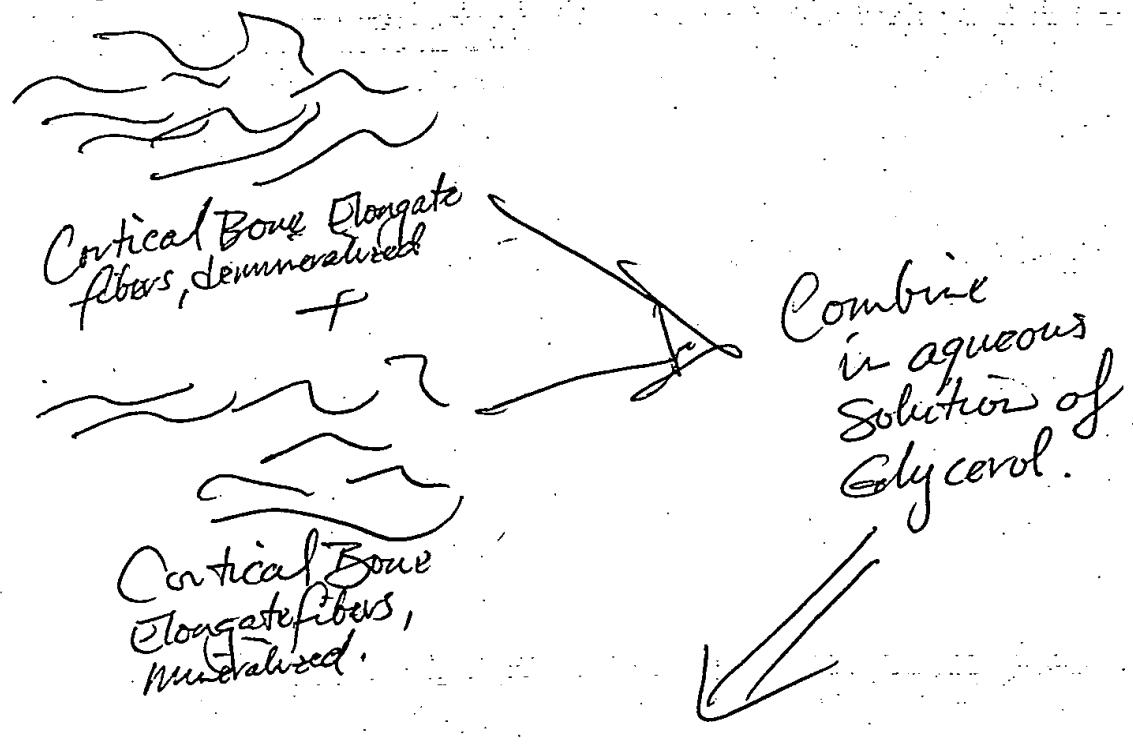


Appearance
through
Cross-section:

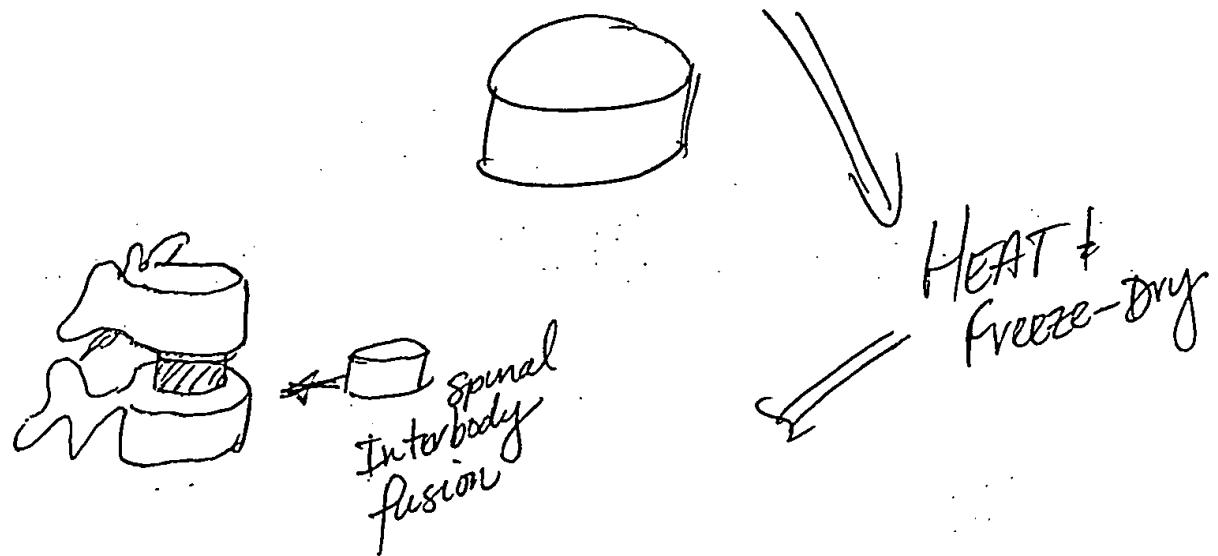


Machined
to shape -
(Parietal
Bone - portion)

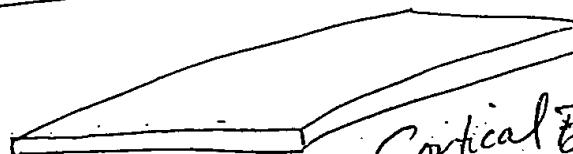
285-79 EXAMPLE 2:



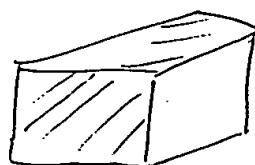
Press into pellet.



285-79 EXAMPLE 3:

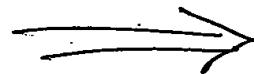


+

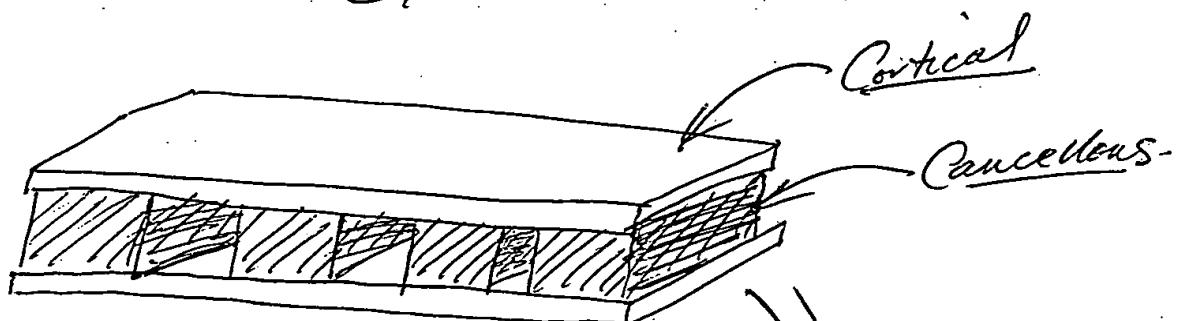


Cortical Bone Strip

Surface Demineralize



Clamp in place
Cross-link



Cortical

Cancellous

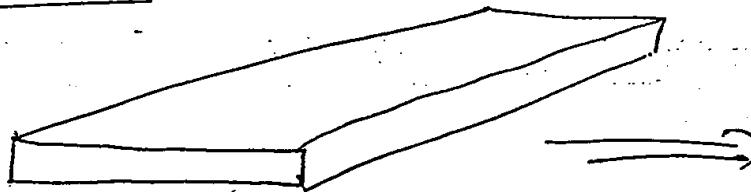
Cut & Shape



used as a spinal
onlay graft
for ~~posterior~~ posterolateral
intertransverse process fusion.

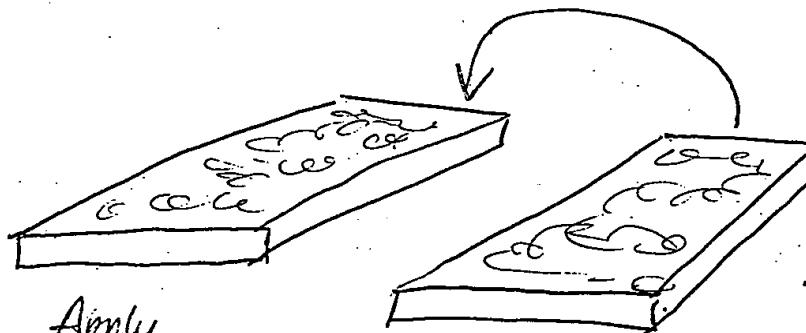
285-79

EXAMPLE 4 :



Cortical Bone Slices

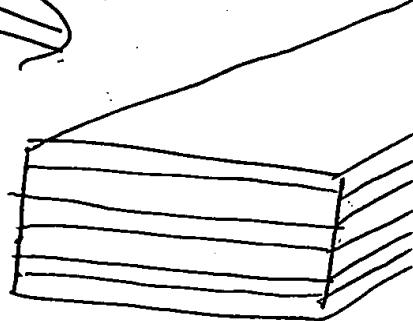
Surface Demineralized
with Acid, Wash.



Apply
Tissue Transglutaminase

Apply CaCl_2
Solution.

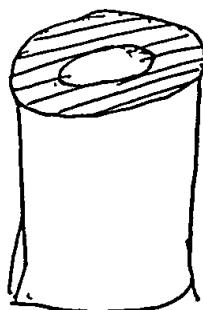
Then assemble with treated
surfaces touching



Machine
to shape.



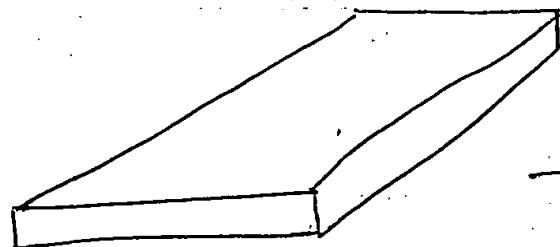
Femur
(Huge Bone)



Long bone
Segment
replacement

285-79

EXAMPLE 5:



Cortical Bone
slice

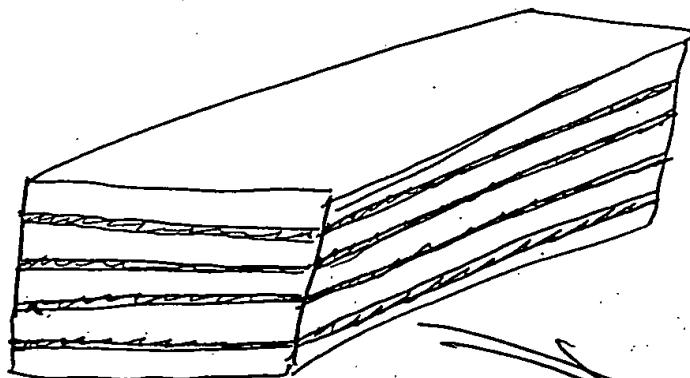
→ Surface
Deminerelize
with Acid

+



Cortical
Bone Powder.

→ Fully Deminerelize
with Acid.



Layer with
powder both
slices.
Clamp and Cross

Machine to Shape



Proximal
Tibia
Shape.